

Full Length Research Paper

Antibacterial and antioxidant activity of *Justicia spicigera* extracts: Activity enhancement by addition of metal salts

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Justicia spicigera was screened for its phytochemicals. The results showed the presence of alkaloids, flavonoids, tannins, phenols, saponins and steroids. Extracts of *J. spicigera* prepared with solvents of different polarities (n-Hexane, Chloroform, Methanol, and Ethyl acetate) was used to analyze the antibacterial properties in the absence and in the presence of metal salts. The antioxidant activity was analyzed using 2, 2-diphenyl-1-picrylhydrazyl reagent (DPPH). Quantitative determination of total phenolics was carried out using spectrophotometric method. The results indicate that the tested bacterial strains were most sensitive to the n-Hexane extract but moderately sensitive to the chloroform and methanol extracts. The chloroform, methanol, and ethyl acetate extracts showed potent radical scavenging activities. The ethyl acetate and methanol extracts showed higher total phenolic content than the other extracts. Antioxidant activity was found in the extract-Fe³⁺ ion combination. The extract-Zn²⁺ ion combination showed enhanced antibacterial activity against tested bacterial strains compared to the extract alone. The results scientifically establish the efficacy of using the plant extracts and its metal salt combination as antibacterial and antioxidant agents.

Key words: *Justicia spicigera*, total phenolic content, antibacterial activity, antioxidant.

INTRODUCTION

Justicia spicigera a shrub from the Acanthaceae family has been found to produce biologically active species that are responsible for the plant therapeutic properties (Domínguez et al., 1990; Euler and Alam., 1982). It is commonly referred to as firecracker plant due to their narrow red flowers, mutile in Mexico and Iyip abasi by the Efiks in Nigeria. *J. spicigera* has been used in

traditional medicine for many years as a stimulant, for gastrointestinal disorders, diabetes, inflammations, blood depurative treatment, uterine cancer, skin and kidney infections (Marquez et al., 1999). The infusion of *J. spicigera* has characteristic red color.

Currently there is much interest in the protection of low density lipoprotein, important cells and organs as well as

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food systems against oxidative damage caused by superoxide, hydroxyl and peroxy radicals. This has led to an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products from plant origin rather than looking for synthetic ones. Antioxidants may guard against reactive oxygen species (ROS) toxicities through the prevention of ROS construction by the prevention of ROS attack, scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological targets to ROS attack (Sen, 1995). Free radicals, ROS, and reactive nitrogen species (RNS) are associated with many pathological conditions such as atherosclerosis, arthritis, ischemia, reperfusion, injury of many tissues, central nervous system, gastritis, cancer and AIDS (Cook and Samman., 1996; Kumpulainen and Salonen, 1999). The anti-oxidative properties of phenolics are due to mechanisms such as scavenging of free radicals, chelation of metal ions, and inhibition of enzymes responsible for free radical generation (Akinmoladun et al., 2007).

Several studies have been made in order to determine the antioxidant properties of plants, especially those used in traditional medicine (Jang et al., 2007; Surveswaran et al., 2007). This study is in support of other studies that have been made to replace synthetic antioxidants that are carcinogenic with natural antioxidants from natural sources.

MATERIALS AND METHODS

The plant samples were collected and identified by comparing with the leaves inflorescence in standard text (Akobundu and Agyakwa, 1998); it was further authenticated by Prof. Nmeregini of Forestry Dept., MOUAU.

Extraction

The extraction was performed according to standard method of (AOAC, 1995). The Methanol extract was extracted using 80% methanol and labeled ME, the Ethyl acetate extract was prepared with Ethyl acetate and labeled (EAE), Chloroform extract labeled CE and n-Hexane extract labeled (n-HE).

Phytochemical determination

The phytochemicals determination was done according to the standard method by Harborne and Harborne. (1998).

Determination of total phenolic content

The total phenolic content was measured by spectrophotometric determination with a modified Folin-Ciocalteu method Tuberoso et al. (2007) using Gallic acid as a standard phenolic compound. The total phenolic content was expressed as Gallic acid equivalent (GAE) in g/100 g of dry wt. of extract. Mixture of water and reagent was used as blank. Sample measurement was done in triplicates and the mean and standard deviations calculated in each case.

DPPH Free radical scavenging activity

The free radical scavenging activity of the plant extracts and extracts-metal ions was evaluated by the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) reagent described by Miliauskas et al. (2004). Radical scavenging potential was expressed as inhibiting conc. IC₅₀ value. This represents the sample conc. at 50% of the DPPH radicals scavenged. The assays were done in triplicate and the results were expressed as mean values \pm standard deviations.

Antibacterial screening

Extracts of the leaves 1g was dissolved in 1ml of dimethyl sulphoxide (DMSO) to get a concentration of 100 mg/ml. This was used as stock solutions for the antimicrobial assay (Okigbo and Omodamiro, 2006). Four representative test microorganisms were used in testing the antimicrobial activity of the leaves extracts of *J. spicigera*– n-Hexane (n-HE), Chloroform (CE), Methanol (ME) and Ethyl Acetate (EAE). The test microorganisms used were *Streptococcus pneumonia*, *Salmonella typhi*, *Escherichia coli* and *Aspergillus niger*. The test cultures were originally obtained from Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria and were maintained in nutrient agar.

The antimicrobial test used the filter paper disc diffusion method (Anderson, 1974). Ciprofloxacin drug 0.1 g dissolved in 1ml of distilled water was used on a separate disc. This served as a standard while the disc with DMSO (100 mg/ml) served as the control. The mean was taken as the diameter zone of inhibition. To increase the synergistic effect the metal salts Fe(III) and Zn(II) ions was combined with the extracts for the antibacterial tests. Aliquot containing solutions with a constant concentration of extract 10equivalent and freshly prepared metal salts ZnSO₄, FeCl₃ solution with a stoichiometric equivalent (1-5 eq) were added to give a final extract-metal ion ratio of 10:01, 10:02, 10:03, 10:04, and 10:05. The obtained product was filtered and collected. The experiment was repeated three times for each extract and the means of these values were recorded.

RESULTS

The results of this work are presented in Tables 1 and 2 and Figures 1 to 3.

DISCUSSION

The phytochemical screening done on all the plant extracts revealed the presence of alkaloids, phenolics, flavonoids in all the samples except n- Hexane. Steroids were present in all the extracts but more in the ethyl acetate extract. Tannins and saponins were found in methanol and ethyl acetate extracts. The results of the total phenolic content of *J. spicigera* is shown in Table 1. There is a positive correlation between the total phenolic content and antioxidant activity of *J. spicigera* with that reported for other plant species of Acanthaceae (Sawadogo et al., 2006; Jang et al., 2007). The total phenolic content of *J. spicigera* ranged from 3.28 \pm 0.01 to 5.65 \pm 0.02 g GAE per 100 g dry wt. of extract with methanol extract richer in phenols than the other

Table 1. Total phenolic content of *J. spicigera*

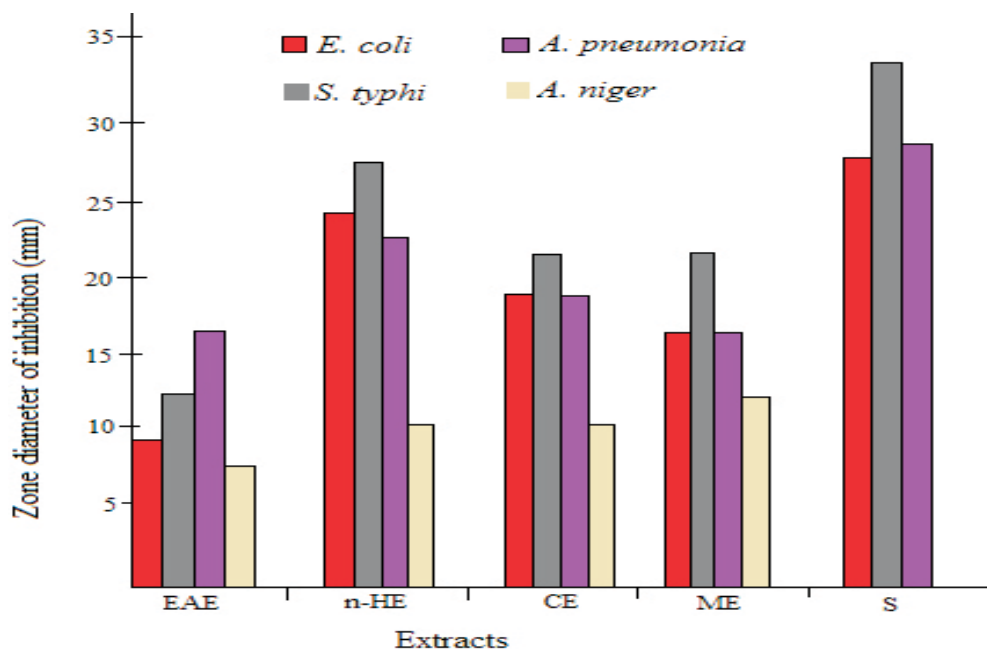
Extract	Total Phenolic content (g GAE per 100g) dry wt. of extract
n-HE	3.28±0.01
EAE.	5.65±0.02
CE	3.99±0.02
ME	4.54±0.04

-Data are means± standard deviations from two extraction replicates.

Table 2. Antioxidant activity of *J. spicigera* with and without metal ion.

Sample	DPPH (IC ₅₀) µg/ml
n-Hexane	34.18±0.50
n-Hexane-Fe ³⁺	34.01±0.15
n-Hexane-Zn ²⁺	36.73±0.80
EAE	13.73±0.21
EAE-Fe ³⁺	11.64±0.18
EAE-Zn ²⁺	12.68±0.39
ME	18.71±0.74
ME-Fe ³⁺	18.01±0.12
ME- Zn ²⁺	18.50±0.19
CE	14.94±0.16
CE-Fe ³⁺	13.28±0.51
CE-Zn ²⁺	14.57±0.13
AA (Ascorbic acid)	14.94±0.30

Data are means± standard deviations from two extraction replicates.

**Figure 1.** Inhibition zone (mm) of extracts against four bacterial strains with standard drug (S= Ciprofloxacin).

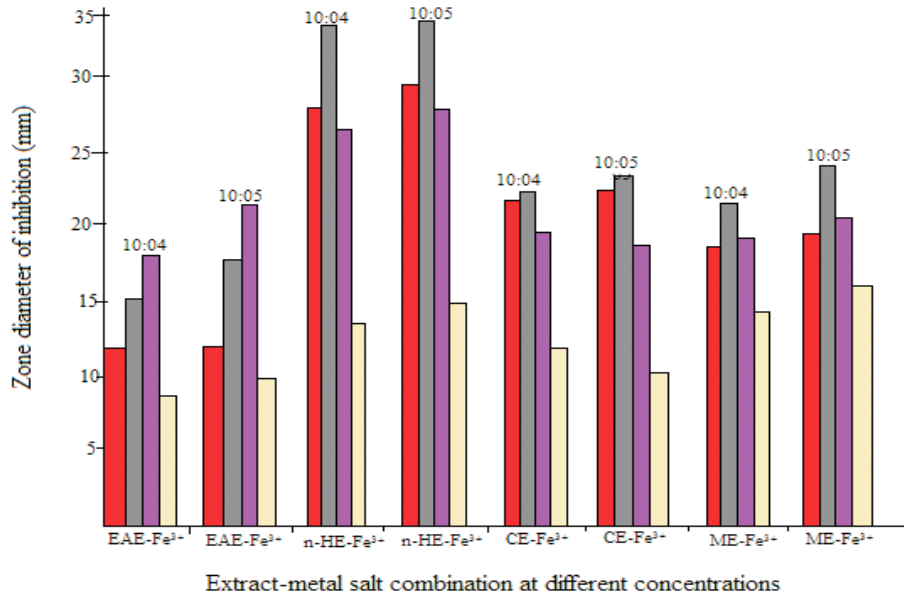


Figure 2. Inhibition Zone (mm) of extract-Fe³⁺ combination against four bacterial strains at concentrations 10:04 and 10:05.

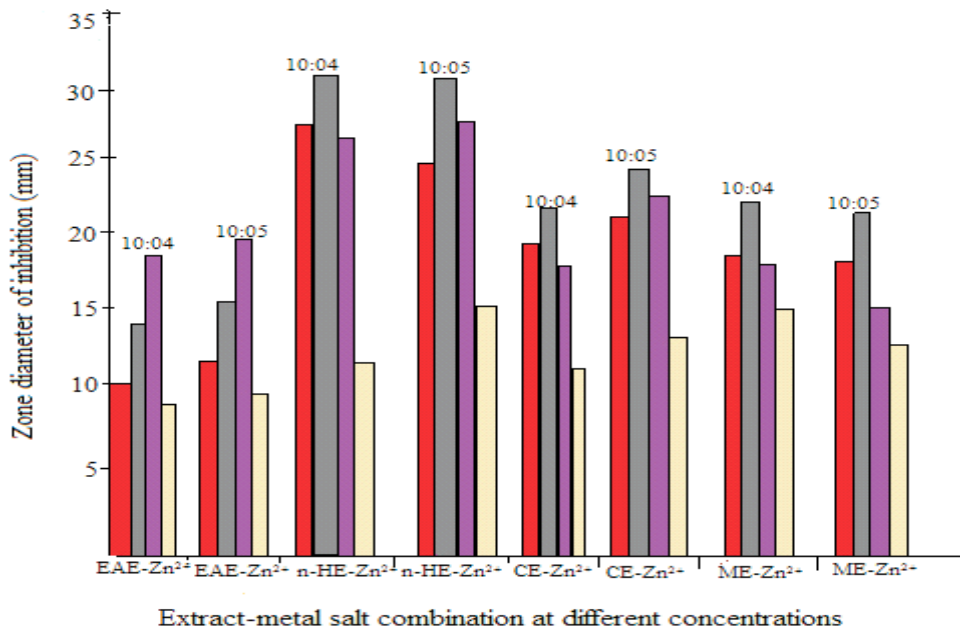


Figure 3. Inhibition Zone (mm) of extract-Zn²⁺ combination against four bacterial strains at concentrations 10:04 and 10:05.

extracts. The results of CE, ME, EAE were found to be significant at $P < 0.05$ but not significant for n- Hexane extract at $P > 0.05$ when compared to the blank.

The results of the DPPH radical scavenging activity of the extracts and ascorbic acid are presented in Table 2. The results show that the extracts exhibited a concentration dependent anti radical activity and the lower IC₅₀ values

are indicative of a higher anti radical activity. At low concentrations (10:01,10:02) the extract-metal ion combination showed results comparable to the extract alone but a discernable effect was observed of the free radical scavenging activity at concentration 10:03. ME-Iron(III) exhibited highest percent inhibition of DPPH radical as compared to other fractions. CE-Iron(III) ion

and EAE-Iron(III) ion exhibited modest scavenging activity than the extracts alone. The results of the radical scavenging activity for the chloroform extract, methanol extract and ethyl acetate extract were found to be significant at $P < 0.05$ but not significant for n-Hexane at $P > 0.05$ when compared to the reference standard ascorbic acid. The extracts-Zn(II) ions did not show significant improvement in radical scavenging activity.

The antibacterial result of the extracts in the absence of metal salts is shown in Figure 1. The results indicate that the tested bacterial strains were sensitive to all the extracts. In comparison the extracts were most sensitive to the n-Hexane extract, moderately sensitive to the chloroform and methanol extracts least sensitive to the ethyl acetate extract. The antibacterial result of extracts with metal salts at different concentrations with Fe^{3+} is shown in Figure 2, the antibacterial results for extracts with metal salts at different concentrations with Zn^{2+} is shown in Figure 3. The results indicate that the extract-metal salt combination enhanced the antibacterial activity than the crude extracts alone. The extract-metal salt combination possessed stronger antibacterial activity against Gram+ve bacteria as compared to the Gram -ve bacteria. This may be because gram -ve bacteria have been reported to be more resistant than Gram +ve bacteria (Russel, 1991). At lower conc. of extract-metal salt combination (10:01; 10:02, 10:03 ratio) no recordable growth was observed. Further increase in metal ion salt conc. to (10:04, 10:05) had significant effect but further increase above that resulted in almost same growth for all tested bacteria. This confirms the study by Simon et al. (2009) which showed that antibacterial properties of natural products can be enhanced by the addition of metal ions due to positive participation of extracts in coordination with the metal ions.

Conclusion

Many plant species are currently used as sources of nutritional additives because of their wide antibacterial and antioxidant properties that increase immunity to some diseases. This study has looked at the biological evaluation of the various extracts of *J. spicigera* and the enhanced synergistic activity by the addition of metal salts. The results suggest that combination of Fe(III) ion with EAE, CE and ME could be considered as a new potential source of natural phenolic antioxidant for food, pharmaceutical, and cosmetic industries. The considerable antibacterial activity of n-HE-Zn(II) ion suggests that *J. spicigera* can serve as selective agent for the maintenance of human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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